or infectious agents, can be used in the methods of the current invention.

Rejection of Claims under 35 U.S.C. 103

The Examiner rejected claims 1-4 as being unpatentable over King. The Examiner indicated that:

> King describes a gene delivery technique in which the gene for gp120 protein of the AIDS virus was incorporated into a plasmid under the control of the CMV (cytomegalovirus) promoter sequence (page 5, abstract). Administration of the expression vector generated both cellular and humoral immune responses in mice to the gp120 protein. While King does not specifically describe a method of immunizing animals, it is stated that the technique is useful in immune therapy and may be an alternative to vaccination in cases of chronic infections and might also be applicable to disease states other than HIV. It would have been obvious to one of ordinary skill in the art, therefore, at the time the invention was made to employ the gene delivery technique in a method of immunizing animals against viral infection with the expectation, barring evidence to the contrary, that the technique would generate specific humoral and cellular immune responses to a variety of antigens.

Applicants respectfully disagree with this assessment.

As amended, the current claims pertain to a method of immunizing a vertebrate animal against an infectious agent by administering a DNA transcription unit that includes DNA encoding an antigen linked to a promoter region, thereby eliciting a humoral and/or cell-mediated immune response, and whereby the animal is protected against disease caused by the infectious agent.

The King reference describes injection of a construct containing the gene for gp-120, a cytomegalovirus (CMV) early promoter sequence and tissue plasminogen activator (TPA) sequence, into the muscle of a mouse, and the resultant production of cytotoxic T cells in the mice against gp-120 protein. However, mice do not develop AIDS

upon being infected with HIV; therefore, production of cytotoxic T cells against gp-120 in mice cannot be used to test for protective immunization. Furthermore, production of cytotoxic T cells does not necessarily indicate that there will be protection upon challenge. One of ordinary skill in the art would not expect that injection of the construct into the muscle of a mouse, as described by King, and the resultant production of cytotoxic T cells in the mice, would lead to protection against disease. It is well known in the art that, even if a candidate fragment of amino acids from a virus may elicit an antibody response to the whole virus, it is necessary to demonstrate that protection accompanies recognition by the antibody response (see Dixon, F.J. and D.W. Fisher, The Biology of Immunologic Disease (HP Publishing Co., New York, 1983), pp. 331-338, and particularly p. 337; a copy of this reference is attached as Exhibit A).

Furthermore, King describes the use of intramuscular injections, and specifically states that muscle injection "side-steps the enzymatic degradation processes which would occur if it were in the bloodstream", i.e., if injection were intravenous. In contrast, the current invention demonstrates the use of several routes of administration, including intravenous, intradermal, intranasal, as well as intramuscular, which one of ordinary skill in the art would not have been motivated to use, given the teachings of King regarding the necessity of intramuscular inoculations.

In addition, King does not teach or describe any data demonstrating that inoculation with a gene for a particular epitope of an infectious agent would prevent infection *in vivo*, or provide antigenic protection against disease upon challenge.

196 C

Rejection of Claims under 35 U.S.C 103

The Examiner maintained the rejection of Claims 15-18 as being unpatentable over WO 90/11092 in view of Huylebroeck et al. Applicants respectfully disagree with this assessment.

WO 90/11092 describes methods of delivering RNA or DNA polynucleotides into a vertebrate cell by interstitial delivery. The levels of protein produced by delivery of polynucleotides were less than 1 μg, as shown in Figure 3 and described in Examples 13 and 18.

Huylebroeck et al. describe use of DNA in cell culture systems to produce the influenza virus HA, which is purified and inoculated into hosts. Huylebroeck et al. also use a recombinant, infectious, replication competent vaccinia and semliki forest virus vectors to express HA. They do not describe vaccination of a host.

The current invention pertains to a method of immunizing a vertebrate, such as a mammal, against an infectious agent, such as influenza virus, by administering a DNA transcription unit including DNA encoding an antigen, such as influenza virus hemagglutinin, linked to a promoter; eliciting a humoral and/or cell-mediated immune response; and thereby protecting the vertebrate against disease.

In order for references to be combined, there must be some teaching or suggestion in the prior art of record supporting the combination (ACS Hospital Systems, Inc. v. Montefiore Hospital, 221 USPQ 929, 933 (CAFC 1984)). However, no such teaching or suggestion appears in either WO 90/11092 or in Huylebroeck et al. The Examiner indicated that:

> because of the increased rate of influenza epidemics which have occurred and are generally well known in the art, much concern has focused on effective vaccines against influenza. It is also generally well known in the art that the major influenza response is

> to the immunodominant hemagglutinin. Thus one would be motivated to employ the influenza viral hemagglutinin in the delivery method described in WO 90/11092 with a reasonabl[e] expectation of success in generating an effective vaccine.

The general knowledge in the art concerning hemagglutinin, and the prevalence of influenza epidemics, without more, is insufficient to support a combination of WO 92/11092 with Huylebroeck et al. Furthermore, neither WO 92/11092 nor Huylebroeck et al. provide the necessary motivation to combine the references. One of ordinary skill in the art would not have been motivated to look beyond the general teachings of WO 90/11092 concerning delivery of polynucleotides, to the teachings of Huylebroeck et al. concerning influenza virus. There is no teaching or suggestion in WO 90/11092 that one of ordinary skill should look to the Huylebroeck et al. reference, which teaches influenza virus in particular, as opposed to looking to a reference describing any other possible viruses or pathogens. WO 90/11092 mentions muscular dystrophy, cystic fibrosis, genetic defects of intermediary metabolism, HIV, Alzheimer's disease, liver and lung disease caused by alpha-1-antitrypsin deficiency, cancers, and controlled release of therapeutic peptides, for example, but does not mention influenza at all.

One of ordinary skill in the art would not have been motivated to look beyond the teachings of Huylebroeck et al. concerning influenza virus, to the teachings of WO 90/11092 concerning the delivery of polynucleotides, because the methods for the production of hemagglutinin differ. First, Huylebroeck et al. use an infectious agent, replication competent vaccinia virus, to achieve high levels of protein expression in an animal. In contrast, the current invention uses purified DNA encoding only the particular antigens, such as hemagglutinin, to produce the immunizing protein in a vertebrate animal. This DNA does

'96 E

not encode replication-competent virus, and is not capable of replication in the eukaryotic host. Second, Huylebroeck et al. utilize cell culture systems to produce the protein for vaccination by direct protein inoculations. They do not teach or suggest that influenza hemagglutinin could be generated in an animal by DNA inoculation, as in the current invention.

In addition, one of ordinary skill in the art would not have been motivated to look beyond the teachings of Huylebroeck et al. to the teachings of WO 90/11092, because of the differences in the amount of protein produced. One of ordinary skill in the art generally uses 10 to 100 micrograms of protein to achieve protective immunizations (see Fields, Orthomyxoviruses Vol. I, pp. 1126-1127, concerning the amounts of protein used in inactivated influenza virus vaccines to obtain protection; a copy of this reference is attached as Exhibit B). In contrast, picogram levels of protein expression were being achieved in WO 90/11092 (see Figure 3). One of ordinary skill in the art would not have been motivated by the teachings of Huylebroeck et al., which address the production of the large amounts of the immunizing protein needed for vaccination by direct protein inoculation, to utilize solely the hemagglutinin DNA to vaccinate using the methods of WO 90/11092, which suggests that directly inoculated DNA produces very low amounts of protein. One of ordinary skill in the art would not have thought that these low levels of protein expression could achieve protective immunizations. Applicants have, for the first time, shown that vaccination by administering a DNA transcription unit encoding a desired antigen, such as hemagglutinin, can result in protection from disease caused by an infectious agent.

Finally, obviousness is established only if the teachings of the cited art would suggest the claimed

196 E

invention to one of ordinary skill in the art with a reasonable degree of certainty of successfully achieving the claimed results. Thus, even if the references were improperly combined, the current invention would not have been rendered obvious, because one of ordinary skill in the art would not have had a reasonable expectation of success in achieving the claimed results. One of ordinary skill in the art would not have had a reasonable expectation that utilization of DNA encoding a particular antigen, such as hemagglutinin, would result in protection of vertebrates against infection and disease, such as influenza. Immune response such as that described in WO 92/11092 is not necessarily indicative of the ability of the vaccine to protect against infection.

The Examiner set forth a new grounds of rejection, rejecting Claims 5-14 as being unpatentable over WO 92/11092 in view of Huylebroeck et al. The Examiner stated that:

> Given the importance of the influenza virus and the importance of the hemagglutinin in the generation of protective immune responses, it would have been obvious to one of ordinary skill in the art at the time the invention was made to combine the teachings of WO 90/11092 on delivery of polynucleotides to vertebrate tissues, with the teachings of Huylebroeck et al. on the construction of non-retroviral expression vectors encoding the influenza viral hemagglutinin, to include the DNA encoding viral hemagglutinin from influenza in a DNA transcription unit. A method of immunizing an animal including humans with the DNA transcription unit would have also been obvious, with the expectation, barring evidence to the contrary, that the DNA transcription unit would avoid the need to purify the HA antigen before use and the transcription unit would also generate humoral and cell-mediated immune responses when administered <u>in vivo</u>. To administer the transcription unit via the intranasal route would have been obvious given the fact that a natural route of infection for the influenza virus is through the nasal cavity. Combining preparations intended for vaccination purposes with physiologically acceptable carriers and

196 G

excipients is well within the level of skill in the art.

Applicants respectfully disagree with this assessment.

The current invention pertains to a method of immunizing a vertebrate, such as a mammal, against an infectious agent, such as influenza virus, by administering a DNA transcription unit including an antigen, such as influenza virus hemagglutinin, linked to a promoter, by an appropriate route, such as by administration to a mucosal surface; eliciting a humoral and/or cell-mediated immune response; and thereby protecting the vertebrate against disease. The references are described above.

As discussed above, in order for references to be combined, there must be some teaching or suggestion in the prior art of record supporting the combination; however, no such teaching or suggestion appears in either WO 90/11092 or in Huylebroeck et al. The "importance of the influenza virus" and the knowledge of the role of hemagglutinin in the generation of protective immune responses, without a specific teaching in either WO 90/11092 or Huylebroeck et al. that would motivate one of ordinary skill in the art to combine the references, is insufficient to support a combination of the references.

Even if the references were improperly combined, the current invention would not have been obvious to one of ordinary skill in the art. As indicated above, obviousness is established only if the teachings of the cited art would suggest the claimed invention to one of ordinary skill in the art with a reasonable degree of certainty of successfully achieving the claimed results. However, one of ordinary skill in the art would not have expected that immunization using the methods described in the application would, in fact, provide protection against disease. First, uptake of the DNA by the cells would be expected to be very

inefficient; even if it is taken up, it could be degraded by the cell and not expressed. Finally, the minute amounts of protein produced would not have been expected to be sufficient to achieve protection against disease.

The Examiner further indicates that "a method of immunizing an animal including humans with the DNA transcription unit would have also been obvious, barring evidence to the contrary, that the DNA transcription unit would avoid the need to purify the HA antigen before use and the transcription unit would also generate humoral and cell-mediated immune responses when administered in vivo". The Examiner refers to language in Huylebroeck et al., where the authors indicate that the recombinant virus which will be used for injection (for immunization) "overcomes the need for purification of the HA-antigen" (p. 284, first full paragraph). Applicants respectfully submit that purification of the antigen is not relevant to the current invention. As described above, the relevant issue is whether a sufficient quantity of protein is produced to generate a protective immune response, not whether the protein is purified. One of ordinary skill in the art would not have had a reasonable expectation that such small levels of protein could produce a protective immune response.

The Examiner also indicates that "to administer the transcription unit via the intranasal route would have been obvious given the fact that a natural route of infection for the influenza virus is through the nasal cavity". Applicants respectfully disagree with this assessment. The mechanism by which a virus enters the body differs substantially from the way in which DNA is taken up into the cells. Viruses attach to host cells via specific receptors, and then enter; most or all of the virion crosses the plasma membrane into the cell during entry. For more detail, see General Virology 3rd ed., (Luria et

al., eds.) 1978, pp. 275-302, a copy of which is attached as Exhibit C). In contrast, "naked" DNA transcription units such as those of the current invention are very inefficiently taken up by the cell by an as yet unknown mechanism. Thus, it would not have been obvious to one of ordinary skill in the art that use of an intranasal route to administer the DNA transcription unit would be effective, given the different mechanisms by which viruses and DNA enter cells.

Conclusion

In view of the amendments and the arguments presented above, applicants respectfully request that the Examiner reconsider and withdraw all rejections.

If the Examiner believes that a telephone conversation will expedite prosecution of this application, the Examiner is requested to call Applicants' Attorney at (617) 861-6240.

Respectfully submitted,

Elizabeth W. Mata

Elizabeth W. Mata
Attorney for Applicants
Registration No. P-38,236
Telephone: (617) 861-6240

Lexington, Massachusetts 02173
Dated: September 1, 1994

176 J